A STUDY OF SOME EFFECTS OF GAMMA RADIATION ON THE ADULTS AND EGGS OF AEDES AEGYPTI

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The studies to be reported in this paper were undertaken to evaluate some of the biological effects of ionizing radiation on the mosquito *Aedes aegypti*. This phase of the work describes in particular the effects produced by gamma radiation on the fertility and reproductive capacities of the males and females of this mosquito species, detailing as well, additional studies on the effects of radiation on the viability of eggs in various stages of development or age. It may be of interest to note that although there is an extensive literature on irradiation of insects, and in particular on irradiation of *Drosophila*, nevertheless, these studies have been concerned in the main with genetic effects rather than with the specific biological effects reported here.

Materials and Methods

The strain of *A. aegypti* used in these experiments was obtained originally from the U. S. Bureau of Entomology and Plant Quarantine Laboratory of Beltsville, Maryland, in June, 1945. It has been maintained since that time in this laboratory in wire screened cages, measuring 27 × 24 ×24 inches, at a constant laboratory temperature of 80° F. and a relative humidity of 75 per cent. To maintain egg production the females have been allowed to gorge on guinea pigs once a week. The resulting eggs have been collected on strips of filter paper, and then conditioned by storing the wet strips in closed jars for a period of three days, after which they are dried and stored at room temperature for future use. The larvae from hatched eggs have been reared in glass jars containing approximately 2500 ml. of tap water, and have been fed on guinea pig pellets added in appropriate amounts each day. On this regimen, it usually requires 8 days for newly hatched larvae to reach the pupal stage. All the adults and eggs exposed to gamma radiation were derived from this colony.

The adults used for experimental purposes were kept in plastic cylinders of 3 inches height and 4 inches diameter, in groups of 40 males and/or 40 females, and were fed during the course of the experiments on 4 per cent sugar solutions. Experimental groups which require blood were fed exclusively on chicks, usually weighing about 300 grams. Adult mosquitoes were kept in the same cylinders during exposure to radiation, and it was possible to expose 6 cylinders simultaneously in the cobalt 60 irradiator used for these experiments. The strips of paper holding the eggs were placed in Petri dishes, and following exposure to radiation were stored

[1] The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

in the same dishes. To insure a maximal hatch, irradiated eggs were kept immersed in water for at least 72 hours.

The mosquitoes were exposed to gamma radiation at an approximate rate of 450 r per minute in air, in a cobalt 60 irradiator of 4 pi geometry.

Results

Irradiation of the male

In the first series of experiments designed to determine the effects of varying dosages of gamma radiation on male *A. aegypti,* 40 male and 40 female mosquitoes were allowed to emerge separately into plastic cylinders and on the fourth day following emergence, the males were exposed to gamma radiation in the cobalt irradiator. On either the first, the eighth, or the fifteenth day following exposure to radiation, the males were then placed in the cylinders containing the females, and shortly after pairing the females were allowed to gorge themselves on normal chicks. The paired mosquitoes then remained together for the duration of the experiment. Control groups were handled in exactly the same manner except that they, of course, received no radiation.

At each dosage level, two to four trials were conducted for both the experimental and control groups, and the number of eggs laid by each group and the percentage of the eggs which hatched were estimated as accurately as possible. Following hatching, the larvae from the experimental groups were grown in the usual manner to determine whether or not they would develop into viable adults. If adults finally developed, these, in turn, were given a blood meal and allowed to mate and the resulting eggs were immersed in water as a final test of parental fertility. Finally, many of the male mosquitoes were dissected at suitable intervals following exposure to radiation in order to determine the presence and condition of the spermatozoa in the seminal vesicles. At the same time, non-irradiated females which had been paired with irradiated males were also dissected to determine the presence and condition of spermatozoa within the spermathecae.

Table I summarizes most of the data concerning the effects of gamma radiation on male *A. aegypti.* In dosages ranging from 1000 r to 30,000 r, exposure to radiation produced no significant effect on the number of eggs laid in those groups in which the males were irradiated one day before they were mated to normal females. Both the control, or non-irradiated animals, and the experimental ones deposited about the same total number of eggs. In the groups in which the males were irradiated but were not paired and offered a blood meal until 8 days after radiation, however, there was evidence of some reduction in the number of eggs deposited among those in which the males had received 30,000 r, while among the groups paired and given a blood meal 15 days after the males had been irradiated, it required only 20,000 r to produce a significant reduction in the number of eggs deposited by the females. In these groups the pattern of egg-laying activity resembled that of 5-day-old and 19-day-old virgin females, allowed to take a blood meal. Such non-inseminated females produced about 20 per cent less the number of eggs than that produced by inseminated females of comparable age, and in addition, the oviposition period of such females extended over significantly longer periods of time.

TABLE I

The oviposition pattern of normal female Aedes aegypti mated to males exposed to varying doses of gamma radiation

Dosage (r)	Days post-irradiation, mating and blood-meal								
	1			8			15		
	No. eggs laid	Length oviposition period (days)	Per cent hatch	No. eggs laid	Length oviposition period (days)	Per cent hatch	No. eggs laid	Length oviposition period (days)	Per cent hatch
0	1060	11	100	1250	13	100	1100	16	100
1,000	1440	12	84	900	10	95	950	9	98
2,500	940	10	64	1150	12	85	1400	13	46
3,500	880	14	43	1000	13	46	1200	17	38
5,000	1150	12	5	1050	13	8	1600	26	9
7,500	1110	9	5*	1230	11	6*	1400	21	2*
10,000	1530	14	1	1000	14	2	1150	25	2
20,000	900	14	0	1100	22	0	800	41	0
30,000	1000	11	0	850	40	0	750	57	0

* Fertile F-1 progeny reared.

The length of the oviposition period was markedly affected by both the quantity of radiation applied to the males and the interval between exposure to radiation and mating. In 27 control groups given a blood meal and allowed to mate on the fifth day following emergence, eggs were deposited for periods ranging from 8 to 15 days, averaging 11 days, after the blood meal. In 27 corresponding experimental groups, in which males exposed to doses of radiation ranging from 1000 r to 30,000 r on the fourth day following emergence were mated on the next day and the females then given a blood meal, the length of the oviposition period differed very little from that of the control animals. In a second series, in which the females of 25 control groups were given a blood meal and allowed to mate on the twelfth post-emergence day, eggs were deposited for periods ranging from 8 to 16 days, averaging 13 days, after the blood meal. In the corresponding experimental groups, in which the males were irradiated on the fourth post-emergence day and mated 8 days later, there was no significant difference in the length of the oviposition period among those groups in which the males received radiation dosages ranging up to 10,000 r. At dosages of 20,000 r, however, eggs were deposited up until 22 days following the blood meal, and in the groups in which the males had received 30,000 r, the females continued to deposit eggs for periods as long as 40 days after the blood meal.

Increases in length of the oviposition period were even more apparent in the third set of experimental groups, in which the males and females were paired and the females then given a blood meal 14 days after the males had been exposed to radiation. In this series, 24 control groups in which non-irradiated mosquitoes were mated and given a blood meal on the nineteenth day after emergence, eggs were deposited for periods ranging from 9 to 25 days, averaging 16 days, after the blood meal. In the experimental groups in which the males had received radiation dosages up to 3500 r on the fourth day and mated 15 days later with normal females, the length of the oviposition period was similar to that of the control groups cited above. At dosages of 5000 r to 10,000 r, however, eggs were deposited for periods which averaged 24 days after the blood meal, while at 20,000 r dosages, this period was extended to an average of 41 days, until at 30,000 r dosages the females continued to lay eggs for periods averaging 57 days. It is of interest to note that along with the increase in the egg-laying period, there was a corresponding increase in the number of days in which the females laid eggs. Thus, in the series in which males exposed to 30,000 r were mated to normal females 8 days later, and in which the oviposition period was extended to 40 days, eggs were actually laid during this interval on 27 different days as compared to 9 days on which normal females mated to normal males laid eggs. In the most extreme case, in which males exposed to 30,000 r were mated 15 days later, the females laid eggs on 33 different days as compared to 10 days for the control females in that series.

In view of these results, it was necessary to determine, first, the effects of gamma radiation on the spermatozoa in the male mosquito. For this purpose, males were dissected and the seminal vesicles examined at appropriate intervals following exposure to varying dosages of radiation. At dosages up to 10,000 r, there was no evident effect on motility, nor any evidence of morphological damage to the spermatozoa for at least 40 days following exposure to radiation. Similarly, at dosages of 20,000 r there was no evidence of loss of motility or morphological damage to the spermatozoa for a period of 25 days. After 25 days, however, during which period the mosquitoes began dying from the effects of the radiation, the spermatozoa were found to be in various stages of fragmentation and degeneration. In males exposed to 30,000 r, loss of motility and deterioration of the spermatozoa did not occur until 20 days after irradiation, at which time again the mosquitoes began dying from the effects of the irradiation. With the radiation dosage increased to 50,000 r, spermatozoa remained motile and normal in appearance for only about 10 days, at which time deterioration of the spermatozoa and death of the adults began to occur simultaneously as usual. In generai, therefore, spermatozoa remained normal in appearance and motile for about as long as the mosquitoes themselves were able to survive the various doses of radiation.

Secondly, to determine whether insemination had occurred, and to observe the condition of the spermatozoa in inseminated females, the spermathecae of normal females mated to irradiated males were examined at appropriate intervals after the animals had been paired. As a result, it was found that all the females paired with males one day after the males had been exposed to 20,000 r contained motile spermatozoa normal in appearance for at least two weeks after mating. On the other hand, only about one-half the females mated 15 days after the males had been exposed to 20,000 r contained normal spermatozoa the third day after mating. In the remainder, either the spermatozoa were in fragments or, more usually, there were no spermatozoa present. And again, the spermathecae of all the females mated a day after the males had been exposed to 30,000 r contained normal appearing spermatozoa for at least two weeks after pairing. If, however, pairing was delayed until 15 days after exposure of the males to radiation, none of the spermathecae of the 25 specimens were found to contain spermatozoa when examined the day after mating. Thus, these data suggest that the reduction in egg production and increase in the period of oviposition evidenced when mating was delayed following exposure of the males to radiation was due simply to inability of more and more of the deteriorating males to copulate successfully rather than to any lack of motile spermatozoa.

That morphological integrity and motility of the spermatozoa, or significant changes in the egg-laying pattern of females mated to irradiated male mosquitoes, are inadequate criteria for determining the biological effects of gamma radiation on the fertility of the males, is shown by observations on the hatching of eggs laid by normal females mated to males exposed to varying doses of radiation, and the viability of the larvae emerging from such eggs. Thus, as shown in Table I, although in all the groups mated at various intervals following irradiation of the males, the females continued to lay eggs in considerable numbers, nevertheless, the number of larvae hatching from these eggs become progressively less with increasing exposure to irradiation of the males.

In view of the fact that even under well controlled conditions of temperature and humidity, there was considerable variation in the percentage of eggs hatching from eggs produced from normal matings, it was necessary for purposes of comparison, to consider the hatch from a given number of normal, control eggs as a 100 per cent hatch. And again because of variations in the hatch from a given collection of eggs, differences in the degree of hatch between eggs from control and experimental were not too apparent until the disparity in hatching between the two groups became quite considerable.

As shown in Table I, in all three groups mated at various intervals following irradiation of the males, the first real evidence of any appreciable reduction in the percentage of larvae hatching from a given number of eggs was in those groups in which the males were exposed to at least 2500 r. In the groups in which the males had received 3500 r there was little further reduction in hatch except in the group in which the males were not mated with normal females until 15 days after their exposure to radiation, in which case the hatch was only 18.4 per cent of that in the control group. There was, however, a sharp decrease in the hatch from eggs derived from groups in which the males had received 5000 r, so that in the ones mated 1 and 8 days following exposure to radiation, only 5.3 per cent and 7.6 per cent, respectively, of the eggs hatched, as compared to the controls. On the other hand, as if to illustrate again the variations in hatching which may occur, in the third remaining group which had been mated 15 days following exposure to radiation, 20.0 per cent of the eggs produced hatched into viable larvae.

There was little further reduction in the proportion of eggs hatching among the groups in which the males had received 7500 r. In the groups in which the males had been exposed to 10,000 r, however, only slightly over 1 per cent of the eggs hatched, while in the groups in which the males had received 20,000 r and 30,000 r, no larvae ever hatched from the eggs which were produced.

The fact that the number of viable eggs produced was the same, at any given dosage, in the groups mated 15 days after irradiation as in the groups mated the day following exposure, indicates that once a sperm was damaged to the extent that it was no longer capable of fertilizing an egg, there was no further recovery and the injury remained permanent, whereas if a sperm escaped such lethal injury initially there was no further physiological deterioration and it remained uninjured and capable of fertilizing an egg. Since there was no decrease or increase in the production of viable eggs to indicate either deterioration or recovery, neither of which process could be expected to proceed at the same rate, it appears reasonable to assume that gamma irradiation has a kind of all-or-none effect on the spermatozoa of *A. aegypti,* and that the extent of this effect will depend upon the level of radiation that has been administered. Further, the data cited above lend further support to the assumption that spermatogenesis is not a continuing process during the adult life of the male *A. aegypti.*

It is of interest to note that the larvae hatching from the matings described above could be reared successfully and the resulting adults, when mated themselves, produced fertile, viable eggs, providing there were sufficient larvae present to eliminate the cultivation problems that arise when larval colonies are too small. Thus, from matings in which the males had received dosages up to 3500 r, larvae were reared with comparative ease, but at dosages of 5000 r and 7500 r, when only very few larvae were available, rearing them to adulthood became a major problem of cultivation. Even at these dosages, however, the few larvae that were finally grown mated successfully as adults and produced viable progeny. Although several attempts to rear the isolated larvae hatching from matings in which the males had received 10,000 r were unsuccessful, there is some reason to believe that with enough care such larvae could be grown to adults, and in such a case the adults would in all likelihood produce viable progeny.

Irradiation of the females

In these experiments, designed to measure the effects of varying doses of gamma radiation on the oviposition habits of female *A. aegypti,* again 40 male and 40 female mosquitoes were allowed to emerge separately into plastic cylinders except that in this case on the fourth day following emergence, the females were irradiated and then paired with normal male mosquitoes on the first, eighth, or fifteenth day following exposure to radiation and offered a blood meal soon after they had been mated. The resulting eggs were collected, counted, then incubated as usual in a saturated atmosphere for five days, and finally hatched. The larvae were grown to adults and mated, and the resulting eggs were then collected and allowed to hatch as evidence of fertility of the F-1 adults. It may be noted here that as in the case of eggs produced from matings in which the males were irradiated, if the eggs hatched they could usually be grown to adults.

As shown in Table II, there was no significant reduction in the number of eggs produced by females exposed to 1000 r, or 2000 r, and mated the following day. There was, however, a significant reduction in the number of eggs produced by females exposed to 2500 r, while the females exposed to doses as high as 10,000 r produced only a few isolated eggs. On the other hand, in the groups mated 8 and 15 days following irradiation, although again there was little or no reduction in the number of eggs produced by females exposed to doses up to 2000 r, egg production dropped off sharply in females exposed to 2500 r, while females exposed to 5000 r laid no eggs at all. In general, therefore, the longer the mating of females exposed to radiation doses above 2000 r was delayed, the fewer the eggs they produced, and the lower the dosage required to eliminate egg production entirely. Similarly, the fewer the eggs that were produced, the less the proportion of them that eventually hatched into viable larvae. Thus, whereas 64.9 per cent of the eggs laid by females exposed to 3000 r and mated one day later hatched, only 25.6 per cent of the eggs laid by females exposed to the same dosage but mated 15 days later hatched, and similarly while 67.0 per cent of the eggs laid by females exposed to 3500 r hatched when they were mated 24 hours later, only 24.0 per cent of the eggs hatched when mating was delayed 15 days. And, finally, although a small percentage of the eggs laid by females exposed to 5000 r and mated one day later hatched into viable larvae, none of the females exposed to 5000 r or higher laid any eggs at all when mated 8 or 15 days following exposure.

These data indicate that in female *A. aegypti* exposed to radiation above certain threshold levels, in this case approximately 2000 r, the ovaries are not only incapable of recovering from the injury produced by radiation, but rather that the functional activity of the ovary becomes progressively further impaired during the interval following exposure. It may be noted in passing that unlike the case of normal females mated to males exposed to radiation, in which the reduction in egg production resulted in lengthening of the period of oviposition, in this case the reduced egg production of irradiated females mated to normal males resulted instead in significant lessening of the oviposition period, indicating again the loss in functional activity of the impaired ovaries.

To determine whether irradiated females could mate successfully and whether spermatozoa could survive in them, groups of virgin females were exposed to doses of 20,000 r and 30,000 r, paired with normal males either 1 or 15 days later, and then dissected at suitable intervals following mating. The spermathecae of all the females exposed to 20,000 r, and paired the day following radiation, contained motile spermatozoa for a period of two weeks after mating had occurred. However, when

TABLE II

The oviposition pattern of female Aedes aegypti exposed to varying doses of gamma radiation and then mated, at different intervals following irradiation, to normal males

Dosage (r)	Days post-irradiation, mating and blood meal					
	1		8		15	
	No. eggs laid	Per cent hatch	No. eggs laid	Per cent hatch	No. eggs laid	Per cent hatch
0	1130	100	1070	100	1100	100
1,000	1050	100	1100	100	1000	100
1,500	1350	71	1050	90	1000	91
2,000	1000	87	1000	90	700	78
2,500	700	87	830	62	650	32
3,000	650	65	800	42	400	26
3,500	550	43	600	35*	190	24*
5,000	25	13*	0		0	
7,500	10	0				
10,000	10	0				
20,000	0					

* Fertile F-1 progeny reared.

mating was delayed until 15 days after irradiation, an average of only 8 out of 10 females were found to contain spermatozoa during the ensuing two weeks. Similarly, the spermathecae of all females exposed to 30,000 r contained spermatozoa for at least 2 weeks if pairing took place the day after irradiation, but if mating was delayed until 15 days after exposure to radiation, then only an average of 4 out of 10 females dissected at intervals during the subsequent 2 weeks contained spermatozoa. From these results, it would appear as if females exposed to high doses of radiation are capable of mating shortly after exposure and that spermatozoa will apparently survive in them for at least two weeks, but if mating is delayed too long, radiation injury to the mosquito as a whole progressively reduces the chances of successful mating

The second series of experiments was designed to ascertain whether viability or production of eggs was influenced by insemination of the females prior to radiation rather than subsequent to radiation as in the previous experiments. Accordingly, to assure insemination 40 females and 40 males were allowed to emerge into the same cylinders and were maintained together for four days following emergence. At the end of this period the males were removed and the females were exposed to varying doses of radiation. One, eight and fifteen days after irradiation these females were allowed to take a blood meal and the resulting eggs were collected, incubated and hatched. The data presented in Table III indicate that females inseminated prior to irradiation and given blood at various subsequent intervals laid about the same number of eggs as females inseminated at similar intervals following exposure to radiation, as shown previously in Table II. Similarly, as in the previous experiment, the number of eggs produced decreased significantly at

TABLE III

The oviposition pattern of female Aedes aegypti mated first to normal males, exposed to varying doses of gamma radiation after mating, and then allowed to take a blood meal at different intervals following irradiation

Dosage (r)	Days post-irradiation, blood meal					
	1		8		15	
	No. eggs laid	Per cent hatch	No. eggs laid	Per cent hatch	No. eggs laid	Per cent hatch
0	1300	100	1280	100	1000	100
1,000	1400	75	1300	68	1250	86
1,500	1450	55	1220	68	1320	60
2,000	1350	67	1150	61	900	57
2,500	1180	67	910	33	630	44
3,000	600	34	620	15*	670	14
3,500	380	3	200	0	520	7*
5,000	30	2*	0		0	
7,500	30	0	0		0	
10,000	40	0				
20,000	0					

* Fertile F-1 progeny reared.

2500 r to 3000 r, and except for those females which received a blood meal one day after irradiation, egg-laying was almost entirely inhibited at doses 5000 r or above.

On the other hand, the number of eggs which hatched from among those laid by females inseminated prior to exposure to radiation was consistently below the number which hatched from among those laid by females inseminated subsequent to exposure to radiation. Thus, in the groups in which the females were mated and then exposed to as little as 1000 r there was a significant reduction in the number of eggs hatching from among those produced, while there was no reduction in the number of eggs that hatched of those produced by females inseminated following exposure to the same dosage, whereas 43 per cent of the eggs laid by females inseminated subsequent to exposure to 3500 r hatched out, only 3 per cent of the eggs produced by females inseminated prior to exposure hatched into viable larvae. Obviously, insemination of females prior to exposure to radiation reduces the viability of the eggs simply by introducing an additional source of injury, namely, injury to the spermatozoa in addition to the effect on the ovaries.

As before, it was possible to rear fertile F-1 progeny whenever enough larvae hatched out from the eggs. Thus, fertile F-1 progeny were reared from females inseminated either before or after exposure to doses as high as 5000 r and then given a blood meal the day after radiation. At dosages above 5000 r although a few eggs were produced, as shown in Tables II and III, none hatched. On the other hand, no eggs were produced by females exposed to 5000 r but not allowed a blood meal until 8 and 15 days following exposure to radiation, and fertile progeny were reared only from females receiving 3500 r.

In female *A. aegypti* ovarian activity is apparently suspended until the animal takes a blood meal. Following a blood meal development of the egg proceeds, fertilization takes place providing the spermathecae contain spermatozoa, and finally oviposition begins. At the temperatures maintained in this laboratory, female *A. aegypti* will begin to produce fertile eggs approximately 48 hours after a blood meal has been taken.

The next series of experiments was designed to study the effects of radiation on the cycle of events occurring in fertilized females between the time blood is ingested and oviposition begins. Thus, in these experiments again 40 males and 40 females were allowed to remain together for 4 days following emergence, then at the conclusion of this period, the males were removed and the females were given a blood meal. Subsequently, at intervals of 4, 24 and 42 hours after the blood meal the females were exposed to varying doses of radiation.

The results shown in Table IV indicate that the various doses of radiation had their greatest effect on egg production in those groups irradiated 4 hours after a blood meal, a significantly lesser effect in the groups irradiated 20 hours later, and the least effect in the groups irradiated 42 hours after the blood meal. Thus, whereas the group exposed to radiation 4 hours after it had received a blood meal laid a total of 2305 eggs and no eggs were produced by any females exposed to more than 10,000 r, the group exposed 24 hours after it had engorged laid 12,610 eggs and a few eggs were produced by females receiving as much as 70,000 r. Finally, in the group irradiated 42 hours after the blood meal, 11,010 eggs were laid and it required exposures in excess of 100,000 r to inhibit egg production com-

TABLE IV

Oviposition and egg viability of inseminated female Aedes aegypti exposed to gamma radiation at various intervals following a blood meal

Dosage (r)	Hours post-blood-meal. irradiation					
	4		24		42	
	No. eggs laid	Per cent hatch	No. eggs laid	Per cent hatch	No. eggs laid	Per cent hatch
0	1200	100	1400	100	1480	100
2,500	1060	69	1380	45	1270	29
3,000	600	53	1400	38	1400	13
3,500	230	31	1660	30	1340	7*
5,000	160	7*	1590	10*	1280	1
7,500	110	8	1720	6	1480	0
10,000	40	0	1970	0	1180	0
20,000	0		970	0	980	0
70,000			10	0	310	0
80,000			0		130	0
100,000					10	0
110,000					0	

* Fertile F-1 progeny reared.

pletely. It would appear from these data that the early phases of the complicated sequence of physiological events leading to egg production are highly sensitive to radiation injury but that once the mechanism has been established and has proceeded to some specific developmental stage, oviposition will take place in spite of excessive radiation injury.

On the other hand, the data show that although the mechanisms responsible for egg production become more resistant as development proceeds, the eggs themselves become more sensitive to the effects of radiation as they mature during the pre-oviposition period. Thus, in the groups irradiated 4 and 24 hours following the blood meal, about 8 and 6 per cent, respectively, of the eggs produced by the females exposed to 7500 r hatched into viable larvae but in the groups irradiated 42 hours after the blood meal, only about 1 per cent of the eggs produced by females exposed to 5000 r hatched, while none of the eggs produced by females exposed to 7500 r proved to be viable. And similarly, in the lower dosages, the hatch from eggs produced by females exposed to radiation 4 hours after the blood meal was far more abundant than the hatch from eggs produced by females exposed 42 hours later.

Viable, fertile F-1 progeny were obtained from the eggs produced by females exposed to 5000 r 4 and 24 hours after the blood meal, but the few larvae from eggs of females exposed to 6000 r and 7500 r died shortly before the pupal stage. In the groups irradiated 42 hours following the blood meal, viable, fertile F-1 progeny were reared from the larvae that hatched from eggs deposited by females exposed to 3500 r, but again the few larvae hatching from eggs deposited by females exposed to 5000 r died during cultivation. There is reason to believe that with greater numbers of larvae available, it might have been possible to rear adults from the groups in this experiment in which the larvae died during cultivation. Nevertheless, as before, whenever larvae from irradiated parents could be grown to adulthood, the progeny always proved to be fertile.

Irradiation of eggs

In the first experiments designed to determine the effects of ionizing radiation on the eggs of *A. aegypti,* eggs of two different age groups were selected for study. The first group consisted of eggs that were 25 to 50 hours old in which embryonation had not been completed, while the second group consisted of eggs approximately 400 hours old in which such development had long been completed so that the eggs would normally hatch at once upon immersion in water. In this laboratory, properly conditioned, normal eggs usually require 65 hours from the time they are laid until they hatch. Since the dose rate was about 450 r per minute, the eggs in the first groups were in the cobalt irradiator for periods not above 100 minutes, but the eggs of the second group, which required extraordinarily high doses of radiation to inhibit hatching, had to be kept in the cobalt irradiator for periods up to about 23 hours. Following exposure to radiation, the eggs were kept at insectary temperatures for 1, 8, or 15 days before being allowed to hatch. About 2000 eggs were used for each exposure and there were from 4 to 8 different exposures for each age category. To assure hatching, if hatching was to occur at all, eggs were kept immersed for 72 hours whenever necessary.

The data presented in Table V indicate that the hatching capabilities of the eggs which were 25 to 50 hours old when irradiated, and which have been designated as 2 days old, were far more susceptible to radiation injury than the eggs which were 16 days old when they were irradiated. Thus, whereas in the former it required only 10,000 r to reduce the hatch by 50 per cent, in the latter it required dosages ranging from 20,000 r to 100,000 r to reduce hatching to the same extent. And again, while a radiation dose of 20,000 r sufficed to inhibit completely

Table V

The effect of gamma radiation on the hatch of 2-day-old and 16-day-old Aedes aegypti embryos

Dosage (r)	2-day-old eggs (25–50 hrs.) Days post-irradiation, per cent hatch			16-day-old eggs Days post-irradiation, per cent hatch		
	1	8	15	1	8	15
2,500	100	100	100	100	100	100
5,000	100	100	80	100	100	80
7,500	75	75	75	100	80	80
10,000	50	50	50	80	75	75
15,000	25	25	25	80	75	75
20,000	0	0	0	80	75	50
30,000				75	50	25
100,000				50	20	10
150,000				25	0	0
200,000				10		
500,000				10		
550,000				0		

the hatching of 2-day-old eggs, which were being exposed while embryonic development was still in progress, it required doses ranging from 150,000 r to as much as 550,000 to eliminate entirely the hatching of 16-day-old eggs in which, of course, embryonic development had been completed prior to exposure. In addition, as shown in Table V, it was found that storage of 2-day-old eggs following exposure to radiation produced no further deterioration in the ability of the eggs to hatch but that storage of 16-day-old irradiated eggs resulted in marked deterioration, at the various dosage levels, in the ability of the eggs to hatch successfully. Thus, in 2-day-old eggs, as many eggs hatched out when immersed 15 days subsequent to doses of 10,000 r and 20,000 r as had hatched following immersion only one day following exposure, but with 16-day-old eggs, none of the eggs exposed to 150,000 r hatched out at the end of 15 days although 25 per cent of them had hatched when immersed the day following exposure. And again, although 10 per cent of the eggs which had received 500,000 r hatched when immersed the day following exposure, a comparable hatch was obtained from eggs which had received only 100,000 r when immersion was delayed 15 days following exposure to radiation.

It is of interest to note that although it required enormous doses of radiation to destroy the ability of the larvae to hatch, and there were wide differences between 2-day-old and 16-day-old eggs in the amount of radiation required to produce this effect, nevertheless, fertile F-1 adults could not be produced from either 2-day-old or 16-day-old eggs exposed to more than 2000 r, whether hatched 1, 8, or 15 days following exposure. Thus, only about 50 per cent of the larvae from either 2-day-old or 16-day-old eggs exposed to 1000 r developed into adults, almost all of which, however, were able to mate successfully. From eggs exposed to 1500 r, no more than about 10 per cent of the larvae developed into adults, and of these probably one-half were able to mate. From eggs exposed to 2000 r only about one per cent of the larvae developed into adults, and of these approximately one-third were able to mate and produce viable eggs. The remaining adults in these groups were usually too feeble even to feed and most of them died almost immediately after emergence. However, all the males examined in such cases were found to contain motile spermatozoa. It may be noted, too, that a considerable proportion of the mortality in these groups occurred after ecdysis when the animals, apparently too weak to fly off properly, simply fell back into the water. The larvae developing from eggs exposed to 2500 r died during either the fourth larval instar or the pupal stage, while very few larvae from eggs exposed to 3000 r survived beyond the third instar, but of those that did, all died during the fourth larval stage.

Finally, experiments were designed to determine the effects of varying doses of radiation on the hatching ability of eggs of *A. aegypti* of various ages. For this purpose the eggs were kept at room temperature for periods ranging from less than 24 hours up to 180 days, exposed to radiation and then immersed for hatching 4 days after having been irradiated. Immersion had to be delayed 4 days in order to insure embryonation of the eggs which were irradiated while they were still less than 65 hours old. About 2000 eggs were used in each trial and every group of irradiated eggs was matched by a control, or non-radiated group of eggs from the same adult colony. The experiment was terminated with 180-day-old eggs, since it was found that the hatching of control eggs was so poor after that period that it was almost impossible to isolate any effect of radiation. No hatch whatsoever could be obtained from 270-day-old control eggs.

It is evident from the data presented in Figure 1 that resistance or susceptibility to radiation was related to the age of the egg. During the first 24-hour period following oviposition, a time of active embryonic development, the eggs were particularly susceptible to radiation damage, so that as little as 800 r caused a 50 per cent reduction in hatch while 6000 r inhibited hatching entirely. By the time the eggs were 48 hours old and embryonic development was nearly complete, however, their resistance had increased so markedly that it required 7500 r to produce a 50 per cent reduction in hatch and 25,000 r to eliminate hatching entirely. During the period in which hatching would have normally occurred (third day), resistance was still further increased so that it required 30,000 r to effect a 50 per cent reduction in hatch. Resistance to radiation increased to maximal dosage levels in four- and five-day-old eggs so that it required 75,000 r to produce a 50 per cent reduction in hatch, and at least 130,000 r to inhibit hatching completely. Further aging gradually lessened resistance, however, until by the 180th day after oviposition, administration of 4000 r reduced the hatch by 50 per cent although it still required some 75,000 r to eliminate hatching completely. Progeny were reared from eggs of various ages irradiated at dosages ranging from 1000 r to 2500 r and there was no discernible evidence to indicate that the age of the egg influenced in any way

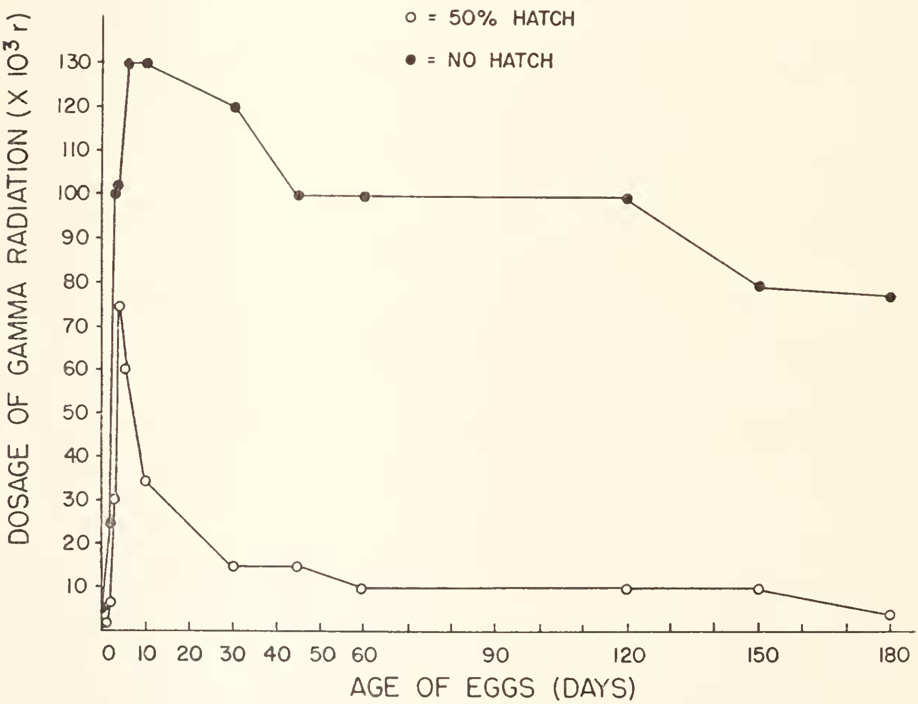

FIGURE 1. The relation between the radiation dosage required to inhibit hatching and the age of the eggs of *Aedes aegypti*.

the viability of the F-1 progeny. Attempts to rear fertile progeny from these irradiated eggs produced results almost identical to the results obtained with 2-day-old and 16-day-old eggs except that in this case, after numerous attempts, a few adults were finally obtained from eggs exposed to 2500 r, which did mate and lay fertile eggs. Thus, again, although the dosage required to impair the impetus to hatch varied with the age of the egg, the dosage required to destroy the viability of the egg was constant regardless of age. It is of interest to note, again, that the dosage was directly related to the length of time larvae developed or survived following exposure to radiation, in that the higher the dosage the quicker larval development was arrested and the larvae died.

Summary

1. It has been shown that when normal *A. aegypti* females are mated and given a blood meal 24 hours after exposure of the males to gamma radiation in doses up to 30,000 r, egg production is not significantly affected. If, however, mating is delayed 8 or 15 days following irradiation of the males, egg production decreases and the period of oviposition increases apparently because fewer males are able to copulate even though they still contain motile spermatozoa.

2. Although eggs continue to be produced in some quantity whether mating is immediate or delayed, fewer larvae hatch from eggs produced by females mated to males exposed to 2500 r, while a very few larvae hatch from eggs produced from matings in which the males received 10,000 r. However, it was possible to grow larvae successfully to fertile adults capable of mating and producing viable eggs only from matings in which the males had received a maximum of 7500 r.

3. It has been shown, too, that the egg production of female mosquitoes, exposed first to gamma radiation and mated 24 hours later to normal males, is significantly reduced among those receiving 2500 r, and almost entirely eliminated among those exposed to 10,000 r. However, larvae which could be grown to fertile adults were obtained only from eggs produced by females exposed to a maximum of 5000 r. When mating was delayed, no eggs were produced by females exposed to doses in excess of 3500 r, although viable larvae hatched from eggs of females exposed to 3500 r.

4. Females inseminated prior to being exposed to radiation produced approximately the same number of eggs at the various dosage levels as females inseminated subsequent to exposure. However, significantly fewer larvae hatched from these eggs than from the eggs laid by females inseminated subsequent to exposure.

5. To determine the effects of radiation during the cycle of egg development which occurs in *A. aegypti* following a blood meal, inseminated females were exposed to gamma radiation at various intervals following engorgement. It was found that egg production was almost entirely inhibited in females exposed to 10,000 r 4 hours after the blood meal, whereas it required in excess of 100,000 r to inhibit egg production in females in which exposure had been delayed 42 hours after the blood meal. On the other hand, although it required higher and higher doses of radiation to inhibit egg production the longer irradiation was delayed, nevertheless, the eggs became more and more sensitive to radiation as they matured within the body. Thus, whereas viable larvae resulting in fertile adults developed from eggs produced by females exposed to 5000 r 4 or 24 hours after the blood

meal, viable larvae could be obtained from the eggs of females exposed to only 3500 r when exposure was delayed 42 hours.

6. Finally, it has been shown that although the dosage required to inhibit hatching of the eggs of *A. aegypti* exposed to gamma radiation varied enormously according to the age of the egg, nevertheless, eggs exposed to doses in excess of 2000 r, regardless of age, could not be grown to adults. Again, however, as in the experiments in which either males or females were exposed to radiation, whenever larvae could be grown successfully to adults, the resulting adults proved to be fertile and capable of producing viable eggs if they were physically capable of mating.